

The role of biomarkers in early prediction and molecular mechanisms of preeclampsia

Iyshwarya Bhaskar Kalarani , Ramakrishnan Veerabathiran ,
Vajagathali Mohammed 

Human Cytogenetics and Genomics Laboratory, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Tamilnadu, India

Abstract

Preeclampsia (PE) is defined as new onset hypertension after 20 weeks of gestation with evidence of maternal organ or uteroplacental dysfunction or proteinuria. PE is a leading cause of maternal death, with about 55000 deaths worldwide each year. Toxic substances that damage the maternal vascular endothelium induce PE, resulting in liver and kidney malfunction. It is crucial for obstetricians to identify as early as possible the patients who are at risk for PE. Polycystic ovarian disease, sleeping disorders, urinary infections, periodontal disease, smoking, lifestyle and familial history of PE are the major risk factors involved in this disease. VEGF, sFlt1, sENG, PAPP-A, inhibin A and activin A proteins, fetal hemoglobin, heat shock protein and placental protein have been helpful in predicting or diagnosing PE and in understanding its pathogenesis. In addition, a better understanding of PE pathogenesis would aid in identifying the most effective treatments that do not impair the fetus' prognosis. The aim of our study is a review of the pathophysiology and biomarkers, such as pro- and anti-angiogenic substances, that may be useful in the detection of PE in the future.

Keywords: risk factors · biomarkers · angiogenic factors · preeclampsia

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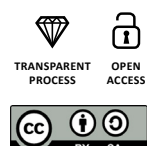
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Corresponding author:

Ramakrishnan Veerabathiran, Human Cytogenetics and Genomics Laboratory, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Tamilnadu, India
e-mail: rkgenes@gmail.com

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Introduction

Gestational hypertension is a condition that increases blood pressure by > 140 mmHg in the systolic and > 90 mmHg in the diastolic for the first time 20 weeks into the pregnancy without proteinuria. Preeclampsia (PE) is defined as new onset hypertension after 20 weeks of gestation with evidence of maternal organ or uteroplacental dysfunction or proteinuria. Additionally, both the mother and the child may develop diabetes mellitus or cardiovascular illnesses later in life due to PE. There are two types of PE: early onset (symptoms appearing before 33 weeks) and late-onset (symptoms appear after 34 weeks) [1-2]. Although early and late-onset PE is related to some risk factors and overlaps in presenting symptoms, their effects on both mother and child can differ [3]. Placental perfusion problems cause proteinuria, edema, and multiple organ failure. Clinical signs range from mild to severe hypertension. Preeclampsia symptoms include cephalgia, impaired vision, light sensitivity, weariness, emesis, upper right abdomen pain, dyspnea and contusions. Normal pregnancy alters the patient's immune system, allowing her to withstand illnesses. Because the fetus expresses paternal antigens, a pregnant woman's immune regulatory competence is critical for a safe pregnancy [4]. In PE, genetic and environmental factors contribute to the aetiology and pathophysiology of the disease. The delivery of the fetus and placenta is recognized as the only effective treatment [5].

PE is the leading cause of maternal mortality, accounting for around 55000 fatalities globally each year [6]. It has a complex etiology with early family-based research showing a role for maternal, fetal and paternal genetic factors [7]. We expect the global prevalence of preeclampsia to range from 5% to 14%, with developing countries experiencing a 4% increase. The total prevalence of preeclampsia varies between studies and populations, ranging from 4% to 23% (4% of pregnancies in the United States, 4.74% in Ethiopia, 6.1% in Kenya and 2-14% in Nigeria) [8]. According to the WHO, PE is common in middle-income developing nations such as India, with a 15% incidence rate and more cases in that country's northern part than in southern. Tripura, the Indian state with the highest PE prevalence, has demonstrated reduction of risk factors such as smoking and multiple pregnancy terminations. Climate change is also a key risk factor in India, with preeclampsia being more common in the summer and spring. Exposure to sunshine, variations in vitamin D deficiency cigarette consumption, lifestyle and ethnicity are the leading causes of hypertension in India [9-10].

According to the 2019 National Institute for Health and Care Excellence (NICE) guidelines, a pregnant woman with a history of autoimmune disease, diabetes or kidney disease has an increased risk of PE. Various risk factors, including nulliparity, older age, chronic hypertension and gestational

diabetes, have been identified as contributing to the high risk of PE. In addition, women with PE have a higher chance of having cardiovascular, kidney or neurological illness later in life, affecting the child's future health [11-12]. Additional clinical factors that enhance the risk of PE include sleep apnea, polycystic ovarian syndrome and infections (including urinary tract infections, periodontal disease and helicobacter pylori). Changing one's lifestyle can reduce PE, e.g. a vegetable and plant-based diet might be linked to a lower incidence of preeclampsia [13]. The aim of this study was to review the pathophysiology and biomarkers, such as pro- and anti-angiogenic substances.

Material and methods

The electronic databases such as PubMed, EMBASE and Cochrane Library were searched for articles published in the years 2010-present. The search terms were: preeclampsia, biomarkers, pathophysiology of PE, risk factors for PE, genetics of PE. We included only the human studies. Papers not written in English were excluded. This is a narrative review, therefore no statistical analysis was performed.

Results

The search retrieved 85 article abstracts. After screening, 26 of them were excluded and we screened the full texts of the remaining articles. A total of 59 articles were included in the review.

Pathophysiology of PE

The pathogenesis of PE is unknown. Currently PE is commonly thought to be a two-stage disease. The first stage is reduced placental perfusion, caused by abnormal implantation and placental vascular development, due to an imbalance in angiogenic factors [14]. The second stage of PE, maternal response, is manifested by widespread inflammation and maternal endothelial cell dysfunction, increased markers of oxidative stress, insulin resistance, reduced immune function and dyslipidemia [15].

Stage 1

Abnormal placentation

During normal placental development, extravillous cytotrophoblast invades the decidual and myometrial spiral arteries [14]. These spiral arteries then lose their endothelial nature and transform themselves from high resistance

vessels into large vessels capable of providing adequate placental perfusion to supply nourishment to the developing fetus. This results in impaired spiral artery remodeling and relative placental ischemia. A trophoblast defect can cause shallow placentation, placental ischemia, insufficient spiral artery transformation and the maternal syndrome of preeclampsia [16-17]. The inability of preeclamptic trophoblast cells to change epithelial cell surface integrin expression to an endothelial phenotype restricts their ability to penetrate. In addition, PE may disrupt the activity of decidual natural killer (NK) cells, which play a role in vascular remodeling [18-19].

The NK cells produce angiogenesis-promoting cytokines and proteins but cannot remodel spiral arteries downstream. Furthermore, PE has been shown to activate circulating T and NK cells. In addition, PE may also occur due to the lysis of HLA-G deficient trophoblast cells caused by the uterine NK cells. Additionally, the absence of trophoblast cells, which should infiltrate the developing spiral arteries, would prevent the placenta from receiving enough oxygen and nutrients. According to recent research, there may be at least one common cause of spontaneous abortion or infertility. This cause is virtually identical to the cause of PE in pregnancies that survive 20 weeks. A recent study indicates that spiral arteries develop relatively narrow, thick-walled, and tortuous vessels in preeclampsia due to the failure of endovascular trophoblast invasions to undergo physiological modification.

Moreover, in PE, trophoblasts do not invade the myometrial segment of spiral arteries, in contrast to a normal pregnancy, in which the transformation of the spiral artery is limited to the decidual region of one-third of the myometrium. Consequently, deep placentation is unsuccessful and the placenta's blood flow is constrained, resulting in insufficient perfusion by the uterus. This phenomenon is associated with PE as well as many other pregnancy complications, such as placental abruption, preterm labor, premature rupture of membranes and intrauterine fetal mortality [20].

Hypoxia-inducible factors

Hypoxia-inducible factors (HIF) 1 α and 2 α , markers of cellular oxygen deficiency, were detected in high quantities in the placentas of PE patients. The production of sFLT1 (soluble fms-like tyrosine kinase 1), a solid anti-angiogenic factor associated with the HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), is inhibited when 2-methoxyestradiol suppresses HIF-1 α , an estradiol metabolite that destabilizes HIF-1 α . Because many variables besides hypoxia control HIF-1 α expression, identifying the dysregulated signal upstream is difficult [21-22]. During placental hypoxia, the transforming growth factor family three (TGF-3) becomes more active, inhibiting the invasion of trophoblasts and the

development of cytotrophoblasts. A natural metabolite of estradiol can decrease placental hypoxia; 2-methoxy estradiol, a natural metabolite of estrogen, reduces placental hypoxia by inhibiting HIF-1 α expression. 2-methoxy estradiol is elevated throughout the 3rd trimester of normal pregnancy [23].

Oxidative stress

A pro-oxidant-antioxidant imbalance favours oxidation, which results in oxidative stress (OS). However, uncontrolled lipid peroxidation can occur in PE, impairing normal endothelial cell function [24]. According to research, PE contributes to placental oxidative stress by causing hypoperfusion and ischemia through remodeling of spiral arteries and cytotrophoblast invasion. The placenta experiences oxidative stress during a normal pregnancy due to increased mitochondrial activity, resulting in excess reactive oxygen species (ROS). However, improper placentation amplifies this impact during preeclampsia [23]. Preeclamptic trophoblasts cultured *ex vivo* produce more ROS, inhibiting the Wnt/-catenin signaling pathway and increasing trophoblast invasion. In addition, anti-angiogenic factors, such as sFLT1, may be expressed [25].

Stage 2

Angiogenic growth factors

Numerous molecules govern the processes of angiogenesis and vascular homeostasis. The VEGF (vascular endothelial growth factor), PlGF (placental growth factor), soluble fms-like tyrosine kinase (sFlt1; fms – feline McDonough sarcoma) and soluble endoglin (sEng) have all been related to the pathophysiology of PE in humans. Endothelial cell activity is required for VEGF, mainly in the liver, glomeruli, and brain, the primary organs affected by preeclampsia. It is a disulfide-linked glycoprotein that is a homodimer involved in angiogenesis and vasculogenesis [26]. The VEGF subfamily functions by interacting with tyrosine kinase receptors, such as EGFR-1, VEGFR-2, and VEGFR-3, that activate cellular responses by phosphorylating the substrate. VEGF and its receptors significantly produced invasive cytotrophoblast in the first trimester of pregnancy for placental vascular development [15]. PlGF is a VEGF family member that plays a role in angiogenesis by binding to VEGFR1/sFLT [27]. It is a powerful angiogenic protein with structural similarity to VEGF that is primarily produced by the placental syncytiotrophoblast. It is also necessary for vasculogenesis during embryonic development in a normal pregnancy. They observed PlGF levels to be lower in PE. Women predisposed to PE had lower PlGF levels, whereas sFlt-1 levels were higher. In PE, sFlt-1 binds to both VEGF and PlGF, inhibiting them from attaching to endogenous receptors. The levels of sFLT1 protein in maternal

plasma or serum were high in preeclamptic placentas [28]. Transforming growth factors (TGF) family members, such as TGF-beta 1 and TGF-beta 3 have a cell surface co-receptor called endoglin. These two substances are powerful inhibitors of trophoblast differentiation and migration. The PlGF, TGF- β and sEng are highly expressed in placental tissue in PE [29].

Angiogenic markers

Soluble Endoglin (sEng)

Cellular endoglin is present on the membranes of vascular endothelium and syncytiotrophoblast cells. It functions as a receptor which inhibits the angiogenic factors TGF- β 1 and TGF- β 3. Its other function is inhibition of nitric oxide (NO) synthase in the endothelial cells, preventing vasodilatory action via the TGF. Several studies show that endoglin has been linked to PE (Figure 1). According to one study, endoglin mRNA and sEng levels were four times greater in preeclamptic women. According to further research, the level of sEng is also used as a prognostic marker. Concentrations of sEng were three-fold higher in patients with mild PE, five fold in severe PE and ten-fold in those with the HELLP syndrome [30]. During PE, placental endoglin is released into the maternal bloodstream, allowing soluble endoglin to become available. A potential mechanism of action is its ability to interfere with TGF- β 1 signaling in the vasculature.

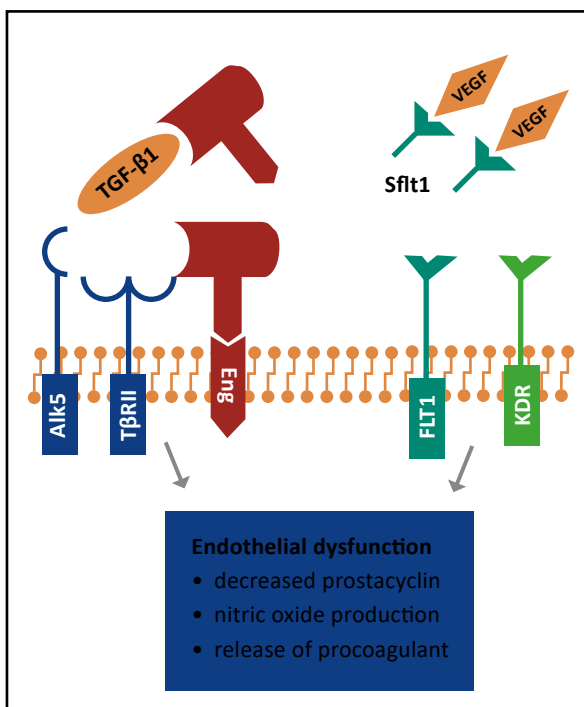


Figure 1. sEng and sFLT-1 cause endothelial dysfunction and leads to preeclampsia

Using adenoviral vectors, over-expression of sEng in rats resulted in improved vascular permeability and mild hypertension without significant proteinuria. Because of over-expressing sFLT1 and sEng with an adenoviral vector, significant vascular damage, nephrotic range proteinuria, severe hypertension, a condition similar to HELLP syndrome, and fetal growth retardation were observed. It is possible, therefore, that sEng and sFLT1 act in different ways to cause endothelial dysfunction and severe PE [31].

Soluble Fms Like Tyrosine kinase-1 (sFLT-1)

The membrane-bound Flt-1 protein (sFlt-1), a shortened splice variant, circulates freely in the bloodstream and binds to PlGF and VEGF. The link between elevated sFlt-1 and PE has been shown in several studies. Levels of sFlt-1 are strongly linked to illness severity, hypertension, and proteinuria [32-33]. In patients with PE, the concentration of sFlt1 rises in the placenta or blood, while PlGF decreases. During the second trimester, sFlt1 and PlGF have shown significant sensitivity in predicting the development of PE (Figure 1) and a high sFlt1:PlGF ratio may also indicate this condition. However, additional clinical research, particularly randomized trials, will be required to determine their utility [34].

Immunological markers

Pregnancy-associated placental protein A (PAPP-A)

The trophoblast produces PAPP-A, a large glycosylated protein thought to function in the implantation process [35]. It enters the maternal bloodstream and binds to the binding protein of eosinophils, inhibiting their proteolytic activity [36]. The first-trimester serum PAPP-A levels are low before other pregnancy problems (e.g. fetal growth restriction, spontaneous miscarriage, placental abruption, premature delivery, gestational diabetes) occur, limiting its use as a PE biomarker (Figure 2). PAPP-A has been proposed as a better diagnostic for fetal growth restriction than preeclampsia [37].

Placental protein 13

Placental Protein 13 (PP13), also known as Galectin 13 (Gal-13) is a polypeptide that combines carbohydrates and is associated with swelling, autoimmune response and cell death. Galectins are vital in controlling certain parts of the genital system in some individuals. It has extreme affinity for sugar residues, namely the ABO blood type antigens AB and B. It may stimulate endometrial arterio-venous dilation and preserve maternal vascular integrity by interacting with glycoproteins and glycolipids [38]. According to studies,

decreased blood PP13 during premature birth may act as a biomarker to identify PE patients. There are some reports that that blood PP13 levels and uterine artery Doppler examination could help to detect individuals with PE before 34 weeks of gestation [39-41]. PE may also be associated with reduced PP13 expression in the placenta [42]. Although PP13 has some predictive significance for PE, Giguere et al needed more data to allow a more precise assessment [43].

Metabolic Marker

Visfatin

It is known that visfatin, a nicotinamide phosphoribosyl transferase enzyme, is produced by adipose tissue that catalyzes the conversion of 5-phosphoribosyl-1-pyrophosphate into nicotinamide mononucleotide [44]. The levels of maternal plasma visfatin were considerably lower in women with PE and it also linked their levels to the severity of the condition [45] (Figure 3). As a result, larger-scale investigations are needed to assess the relevance of visfatin as a possible PE marker.

Endocrine Markers

Inhibin A and activin A

Inhibin A and activin A are glycoprotein hormones that belong to the transforming growth factor-β family. These circulating proteins are primarily obtained from the placenta during pregnancy, and their quantities rise in the third trimester in uncomplicated pregnancies [46]. Inhibin A plays a vital part in the negative feedback of gonadotropins in the endocrine system and Activin A is engaged in various

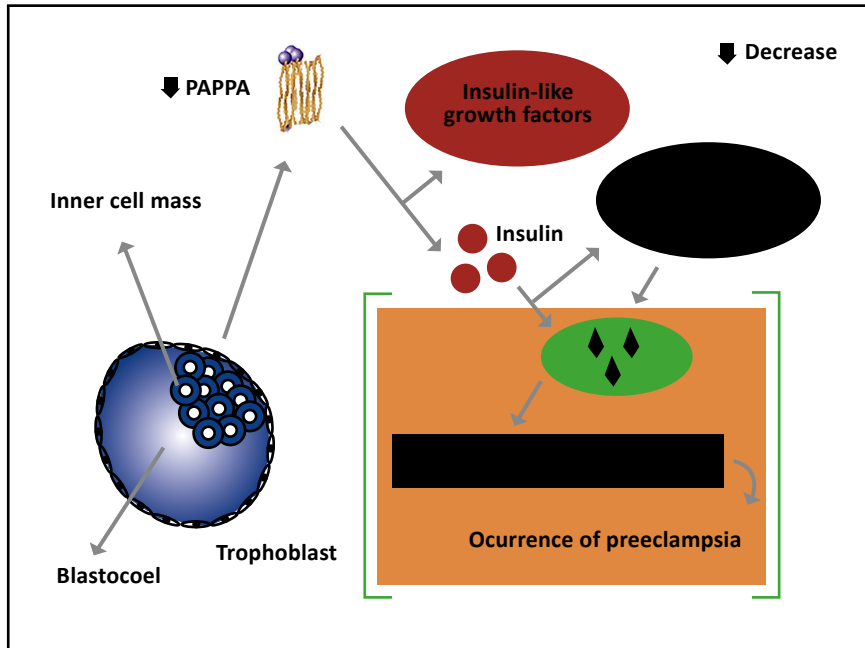


Figure 2. Low levels of the PAPP-A protein and the pathogenesis of preeclampsia

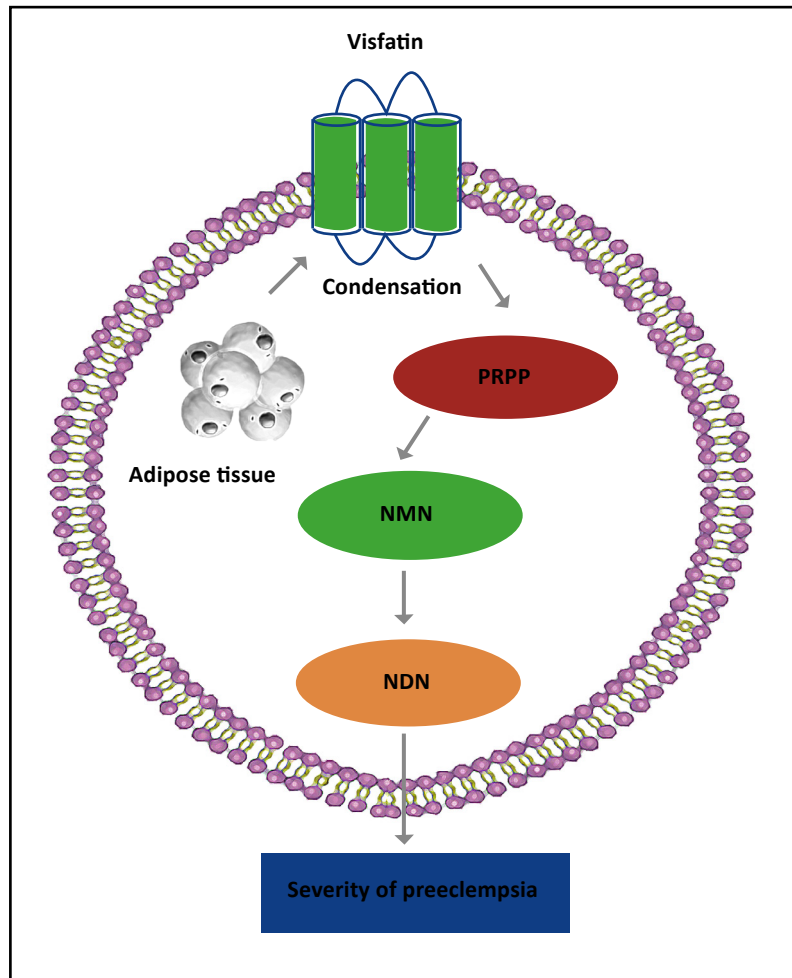


Figure 3. Low-level of visfatin increases the severity of preeclampsia

biological functions. Both hormone concentrations rise in the third trimester of a normal pregnancy and their levels rise tenfold with severe PE. In addition, it causes an increase in maternal oxidative stress and systemic inflammation. Oxidative stress stimulates the production and release of activin A in placental explant and endothelial cells. There are conflicting reports on the increase of inhibin A in the second trimester, which was not as high in PE as Activin A (Figure 4) [47].

Fetal hemoglobin

In PE, fetal hemoglobin (HbF) may provoke oxidative stress. In the placentas and bloodstreams of women with PE, oxidative stress is present. The connection between HbF and PE has prompted researchers to investigate it recently. They have postulated that HbF takes part in PE by causing harm to the placenta, kidneys and other organs, although the actual mechanism remains unknown. Therefore, during PE the placenta has an elevated activity of α 1-microglobulin (A1M), showing that oxidative stress responds to its transcription. HbF causes tissue damage that A1M partially alleviates. There may be a possibility of further research on PE using A1M [48]. The fusion of hemoglobin was increased in the placentas of PE patients due to endothelial injury and inflammation [49]. During oxidative stress, patients with PE

can have HbF released into their bloodstream. Since the HbF serum of PE patients can be higher during premature gestation, it may be a helpful marker [50].

Heat-shock protein

HSP (highly conserved heat shock protein) is typically found in animal/cellular tissues and regulates the cell cycle, immunity and protein synthesis [51]. Concerning the link between HSP and PE, the study focuses mainly on HSP70, which may inhibit apoptosis in various biological processes, including protein function. Maternal adverse reactions, placental disease and oxidative stress can all influence HSP70 expression. A study by Peracoli and colleagues has found that HSP70 may be allied with pro-inflammatory cytokines such as TNF- α , interleukin-1 β , and interleukin-12 [52]. Salt-induced hypertension is an outcome of HSP70 overexpression [53]. Livingston et al. demonstrated that the serum HSP70 concentration in women with severe PE is not higher than usual [54-56]. Because of this contradiction, Saghabi et al. performed a meta-analysis and discovered elevated HSP70 serum levels in PE patients compared to women with normal pregnancies [57]. In addition, Hromadnikova et al. found that patients with PE had elevated levels of HSP70 mRNA. It may be possible to examine HSP70 for PE diagnosis, although most data is from case-control studies and prospective studies are rare [58]. The potential biomarkers' functions and their possible use are mentioned in Table 1.

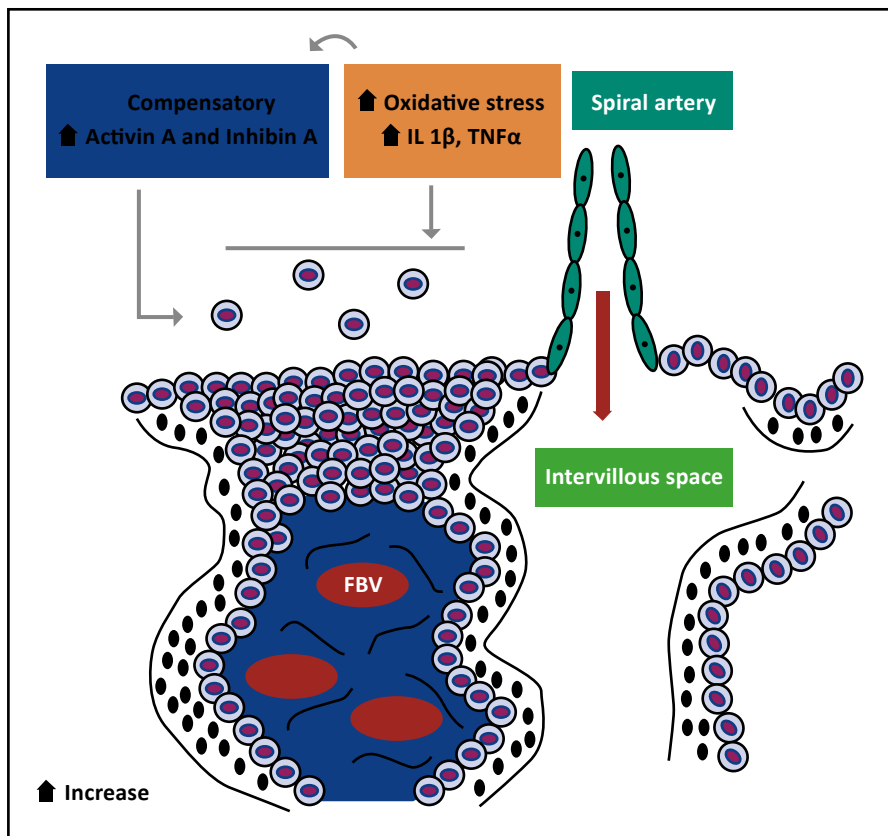


Figure 4. Activin A and Inhibin A are responsible for the manifestation of preeclampsia

Conclusion

Several pregnancy-associated illnesses are associated with PE, which has no known cause. The presence of several protein biomarkers is associated with PE. The following proteins help predict or diagnose PE and aid in the understanding of its pathogenesis: VEGF, sFlt1, sENG, PAPP-A, inhibin A, activin A, fetal hemoglobin, heat shock protein and PP13. Screening for these biomarkers during pregnancy might be clinically helpful. Future research should focus on

Table 1. Biochemical prediction marker for preeclampsia

Biochemical marker	Group	Preeclampsia biomarkers and their potential roles	Potential use
sEng	Angiogenic marker	A protein that inhibits the growth of angiogenic cells	Prognosis/diagnosis
sFIT-1		The placenta is responsible for regulating angiogenesis	Diagnosis
PAPP-A	Immunological marker	A fetus with normal chromosomes probably influences the development of the placenta	Diagnosis
PP-13		A member of the Galectin family, its function remains unknown, but it is believed to induce apoptosis in specific immune cells	Diagnosis
Visfatin	Metabolic marker	It plays a role in glucose homeostasis, and dysregulation of its biosynthesis or signal transduction contributes to disease pathogenesis	Diagnosis
Inhibin A and Activin A	Endocrine marker	It is assumed that inhibition A plays an influential endocrine role in the negative feedback of gonadotrophins. In contrast, activin A is believed to function in various biological tissues	Diagnosis
Fetal Hemoglobin	-	As an antioxidant and heme scavenger, A1M is located in the fetus and is involved in oxygen transport	Diagnosis
Heat shock protein	-	A highly conserved protein that is widely present in organisms and cells and plays a key role in the formation of protein complexes, the regulation of the cell cycle, and the regulation of the immune system	Diagnosis

determining the molecular processes behind the altered angiogenesis and finding new and accurate biomarkers for early disease detection.

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Conflicts of interest

No relevant financial or non-financial conflicts of interests to report.

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